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NEURAMINIC ACID AND CENTRAL NERVOUS SYSTEM FUNCTION
(Neuraminic Acid in the Brain and Tissues of Various Animals)

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ABSTRACT

A comparative study of the sialic acid concentration of the brains and tissues of various animals was made in order to determine whether differences were present which could be correlated with the phylogenetic level of the nervous system.

Sialic acid, probably in the form of N-acetylneuraminic acid (NANA) was present in chordate brains in both the ganglioside, G, and lipid-free residue, R. For G the range was 300-600 ug. % of the total lipids and for R, 350-600 ug. % of the total lipid-free residue. There were no differences which could be correlated with the phylogenetic level of the nervous system.

In animals with diffuse nervous systems whole tissues were analyzed and compared to whole mouse tissue. In whole mouse, sea urchins, and clams the R and G fractions were similar and contained both NANA and N-glycoylneuraminic acid, NGNA. In snails the R fraction contained NANA, NGNA and an unidentified spot with the color characteristics of shikimic and quinic acids. The G fraction contained no NANA. Chitons and sea anemones were similar; the G fraction contained both NANA and NGNA while NANA was missing in the R fraction. All the whole tissues except the sea urchin contained unidentified spots in addition to the NANA and NGNA.

NEURAMINIC ACID AND CENTRAL NERVOUS SYSTEM FUNCTION

(Neuraminic Acid in the Brain and Tissues of Various Animals)

The research carried out under this contract has been concerned with the problems of determining the role of sialic acids (i.e. N-acetyl-neuraminic acid, NANA) in central nervous system function. In an earlier technical note (1) experiments were described in which NANA was injected into experimental animals with no measurable physiological effect. If NANA does have a physiological function which we were unable to measure, it may be that its addition to animals already having a normal concentration of NANA would not be expected to show any physiological activity. A better test animal, then, would be one in which NANA was depleted, lacking or inactivated in some manner. The experiments described here include a comparative study of the sialic acids present in the brains and tissues of various animals in order to determine whether there are species which do not have NANA. It was also thought that the NANA concentration in the brain might be correlated in some manner with the positions of the animals on the evolutionary scale. Such a correlation might provide a clue to NANA function.

PROCEDURE

Whole brains of animals were employed for the phyla Chordata and Arthropoda (Cancer magister). The chordates analyzed were man, cattle, pigs, Long-Evans and Wistar rats, Swiss Webster mice, California white Leghorn chickens, turtles, gopher snakes, grass frogs, and rainbow trout. For larger animals, single, whole brains were used, while with smaller animals it was necessary to pool the brains. The human brain was a mixture of 50 percent white and 50 percent gray matter from a case of subacute sclerotizing leucoencephalitis obtained as a dried sample from G. W. F. Edgar. The brains were removed immediately upon sacrificing the animals and in general were not perfused. A comparison of perfused and non-perfused rat brains showed no significant difference (Table 1). The isolated brains were

then freeze-dried, held in vacuo over P_2O_5 for 48 hours, and weighed again to determine the water content.

The phyla with diffuse nervous systems that were analyzed included the Mollusca: land snails, clams, chitons (Cryptochiton stelleri); Echinodermata: sea urchins (Strongylocentrotus purpuratus); and Coelenterata: sea anenomes (Anthopleura Xanthogrammica). Only the soft tissues of these animals were used except with the sea urchins where only the Aristotle's lantern was used. A whole mouse was analyzed in the same manner as representative of animals with a central nervous system; the tissues were homogenized and then dried as with the brain tissue.

The dried tissues were divided into two fractions according to the method of Svennerholm (2): a lipid soluble-water soluble fraction which includes the ganglioside fraction, G, and a lipid-free residue R. The lipid soluble-water insoluble fraction was discarded. The fractions were obtained by extracting the tissue under reflux for 2 hours with 10 ml. of methanol;chloroform (2:1). The lipid-free residue was then dried in vacuo over P_2O_5 (after removal of the residual organic solvent , weighed, and the lipid calculated by difference.

Some sialic acid analyses were carried out by hydrolyzing the lipid extract directly after removal of the organic solvents (discussed under correction factors). In the final method adopted, 10 ml. of chloroform and 5 ml. of 0.1 percent NaCl were added to the original 10 ml. of lipid extract. The two phases were then mixed thoroughly and separated by centrifugation. After removal of the aqueous layer the residual organic layer was washed twice with 5 ml. portions of chloroform;methanol;0.1% NaCl (3:48:47). The three washes were then combined and evaporated to dryness.

The G fraction was dissolved in 0.1 N sulfuric acid and hydrolyzed for 3 hours at 80° C in a tube heater. Fig. 1, curve A, shows the hydrolysis time curve for rat brain G fraction indicating the three hour maximum. Three hours

was also found to be necessary for porcine and bovine brain, and the remaining samples were arbitrarily hydrolyzed for the same time period.

Up to 120 mg. of lipid-free residue were suspended in 3 ml. of 0.1 N H_2SO_4 (0.15 N for sea urchins and chitons because of the buffering of salts present) and hydrolyzed for 2 hours at 80° C. Fig. 1, curve B, shows the hydrolysis time curve for the R fraction from rat brain. These same conditions were found to be necessary for bovine, porcine, and human brain.

The sialic acid in the hydrolysate was determined by the 2-thio-barbituric acid method of Warren (3). The cyclohexanone layer was read in a Bausch and Lomb spectrophotometer at 550 m μ and 530 m μ , and the curve of Jacoby and Warren (4) was used to correct the results for interfering compounds which form a chromogen with an absorption maximum at 530 m μ .

If rat brain lipid fraction is hydrolyzed directly and then analyzed for NANA, curve A of Fig. 2 is obtained. There is as high as 40 percent interference by the 530 m μ chromogen; if the figures are corrected for this interference curve C of Fig. 2 is obtained.

On the other hand, fraction G showed only 0 to 8 percent interference (curve B of Fig. 2) for the same pool of brains. Therefore, because of the large correction necessary for brain lipid extract, the G fraction was used for all the data presented here. In the G fraction of the crab brain in contrast to all the other brain samples, the interference was 52 percent. (There is some question regarding the accuracy of dissection of the crab brains and possibly other tissue was included.)

The corrections for the R brain fractions ranged from 9 percent to 18 percent except for crab where the correction was 90 percent.

In the whole tissues the corrections were higher. In the G fraction the interference ranged from 31 percent in snails to 79 percent in chitons. The interference ranged from 100 percent in sea anemones to 36 percent for

whole mouse.

Since the main interest was to determine whether NANA was present, it was hoped that paper chromatography would indicate the presence of sialic acids qualitatively and no further work was done to remove the interfering chromogens during the analytical procedure.

NANA was used as a standard. It was prepared according to the procedure of Martensson et al (5) except that the starting material was Fraction IV₁ from human blood serum which contained 1.3 percent NANA. The procedure has been outlined in (1). The crude NANA was crystallized from glacial acetic acid (6) or from methanol (5).

N-glycolylneuraminic acid (NGNA) was prepared from porcine submaxillary mucin according to the same procedure.

The results of the sialic acid analyses on animal brains are given in Table 1 for both the G and R fractions along with water and lipid content. The whole tissue analyses are in Table 2. The results are calculated in three ways: mg. percent of the total lipids or lipid-free residue, mg. percent of the total dry brain, and mg. percent of the total fresh brain. Where applicable, standard deviations are given calculated according to Dean and Dixon's statistical short-cuts for observations on small numbers of samples (7).

After the brain and tissue samples were analyzed, like specimens were pooled and the sulfuric acid removed by barium hydroxide. The samples were then placed on ion exchange resins just as for the standard NANA preparation. These pooled samples were used for the paper chromatography.

Descending paper chromatography was carried out on Whatman No. 1 filter paper in solvent system (EHN), ethanol:H₂O:NH₃(80:20:1). The samples were run individually at a level of 75 to 125 micrograms to determine whether one or more of the sialic acids were present. The samples were then run at a level of 25 micrograms mixed with an equal amount of NANA or NGNA as a further check

on the identity of the spots. A second solvent system (BPH), n-butanol;n-propanol: 0.1 N HCl(1:2:1) was also employed where there was sufficient amount of sample. After drying, the papers were sprayed with thiobarbituric acid after the method of Warren (8). The chromatograms were observed under ultraviolet and visible light and, unless stated otherwise, the samples showed the same color as the NANA and KANA.

The results of the EMN chromatography are presented schematically in Fig. 3 (G Fraction) and Fig. 4 (R Fraction). The spots for NANA and KANA show the range of R_f values obtained in seven experiments (KANA average = 0.33, and NANA average = 0.44)

RESULTS

Man: For the G fraction the results were 178 mg. % NANA for dried brain from a 50-50 mixture of cerebral cortex and white matter. Results corresponding to the G fraction have been reported by other workers for these tissues separately (9, 10, 11, 12). If these results are calculated in the same way as ours they range around our sample (158 mg. % to 302 mg. %).

The R fraction of human brain contained 52% mg. % NANA in the lipid-free residue. Svennerholm (13) has reported that in this fraction he obtained 600 mg. % from the cortex and about half this value from the white matter. Our results on the basis of 50-50 white matter and cortex would be higher than Svennerholm's. Our results agree with Svennerholm in the finding that the R fraction had a higher NANA content than the G fraction. The actual ratio of R NANA/ G NANA was 1.7 for dry brain.

Since the literature reports that KANA is the form of sialic acid in human brain (13, 14) for both G and R fractions, the human sample was not chromatographed.

Beef: The bovine G fraction contained 207 mg. % NANA in dry brain. The total brain NANA was 355 mg. % which is lower than the 465 mg. % reported by Svennerholm for calf brain (15).

Chromatography showed only NANA in the G Fraction. Whether the sialic acid was also in the form of O-acetyl was not determined since this form would be destroyed in the use of the ion exchange columns.

The R fraction contained 148 mg. % NANA in dry brain. In contrast to the human results the ratio of R NANA/ G NANA was 0.7.

In EHN only NANA was evident at the 125 microgram level, but in solvent BPH a spot at the level of NGNA was discernable. The spray technique is supposed to detect 5 micrograms of NANA (8). The questionable presence of NGNA in bovine brain is in contrast to the high percentage, 64 %, in serum and kidney of ox (14).

Pork: The G fraction contained 195 mg. % NANA in dried brain with a total of 332 mg. % NANA, as compared to a total of 446 mg. % obtained by Svennerholm (15). In solvent EHN only NANA was present at the level of 100 micrograms, but in solvent BPH there may have been a spot at the level of NGNA. Considerable tailing in this solvent made it difficult to determine individual spots.

The R fraction contained 137 mg. % NANA for dry brain, with the ratio of R/ G NANA 0.7 as for bovine brain. The chromatography results were similar to those of bovine brain except that there appeared to be more material at the level of NGNA in solvent BPH. These results are similar to those for hog serum (15% NGNA), kidney (14% NGNA) and gastric mucosa (20% NGNA) (14).

Rat: The NANA content of the G fraction was 256 mg. % for dry brain (30% higher than the results of Long and Staples (16) who reported on lipid NANA from cerebral cortex and white matter.

Chromatography of 75 micrograms in solvent EHN showed the presence of NANA, material in the R_f range from 0.2 to 0.3 and a spot at 0.55. In solvent BPH

the tailing was so intense with 100 micrograms that only NANA could be distinguished with certainty.

The R fraction contained 201 mg. % NANA in dried brain with a ratio of R/G of 0.8 which was similar to the bovine and porcine results.

Chromatography of 75 micrograms of R sialic acid in solvent EHN showed NANA, a small spot at 0.33, and also at 0.55

Mouse: The G fraction contained 312 mg. % NANA in dry brain, the highest value among the animals analyzed. The R fraction contained 218 mg. % NANA in dry brain with an R/G ratio of 0.7 (the same as the other mammals, except the human). In solvent EHN at 125 micrograms only NANA was discernable in both fractions. In solvent BPH at 25 micrograms, this was also true.

Turtles: The turtle results were 124 mg. % and 189 mg. % NANA in dry brain for G and R respectively, with a ratio of R/G of 1.5 similar to the human results.

The chromatography results were not clear-cut and because of the lack of material could not be repeated. This is true for both G and R and the results were obtained in solvent EHN only. When 100 micrograms of sialic acid were chromatographed alone, a single spot was obtained with an R_f of 0.33. When the chromatography was repeated with the addition of NGNA two spots were evident, one with the R_f of 0.44 indicating NANA. The question as to whether NGNA is also present above that which was added is unanswered. A porcine sample that was analyzed at the same time as the 100 microgram turtle sample also had an R_f similar to NGNA, but on repeating the porcine sample several times, the R_f was 0.44 indicating NANA. The turtle spot was also elongated so that it is possible that two components were present; however fish sialic acid run at the same time also appeared elongated and was later determined to be only NANA.

Snakes: The results for gopher snakes were very similar to those for turtles both analytically and chromatographically. The G fraction contained 161 mg. % and the R fraction 163 mg. % NANA in dry brain with an R/G ratio of 1.

Both fractions contained NANA, but may also contain NGNA.

Frog: Dry frog brains contained 135 mg. % G NANA and 285 mg. % R NANA. The G fraction was chromatographed at a level of 115 micrograms while the R fraction was chromatographed at 70 micrograms. One spot was visible at 0.33 R_f . In a mixed sample with NGNA only one spot was also visible, but there may not have been enough frog sialic acid in the mixture to be visible.

One pool of frog brains was quite different from the others. There was a great deal of interfering material (50%) in the thiobarbituric acid test which was visible to the naked eye.

Fish: Although it was thought that the sialic acid values in rainbow trout would be the lowest among the chordates this was not the case (G = 185 mg. %, R = 251 mg. % NANA in dry brain). The ratio of R/G was 1.3 as for human brain. In solvent EHN at 125 micrograms, NANA was the only component, and in solvent BPH at 25 micrograms, plus and minus NGNA and NANA, the results were consistent with NANA being the only type of sialic acid present.

Crab: Because of the previously mentioned difficulties in dissection, the results on crab may be in error (G showed 7 mg. and R 14 mg. % NANA in dry brain). Chromatography of 14 micrograms of G and 16 micrograms of R in solvent EHN showed one spot with an R_f of 0.33. In addition there was also a spot in R with an R_f of 0.2.

Table 2 gives the sialic acid content of G and R fractions of whole tissues of animals with diffuse nervous systems. Whole mouse tissue was also analyzed for a comparison. The chromatography results are in Fig. 3 and 4. The chromatograms and the analytical procedure showed much more interfering material even in the G fraction. Although the water extraction excluded a tremendous amount of lipid material that was very colored, there were compounds soluble in the aqueous solution that we did not find in the brains of chordates. All of the interference was not removed by resin chromatography; 2-keto-3-deoxygluconic acid

is one compound which would remain on the Dowex 2 giving the same colored product as the sialic acids.

Mouse: The concentration of sialic acid in the G and R fraction amounted to 23 mg. % and 79 mg. % respectively in whole dry tissue which is much lower than the brain content of sialic acid. Long and Staples (16) studying rats, reported that sialic acids in the G fraction were found in the brain, but not in liver, heart, or small intestine, and very little in lung, skeletal muscle, kidney, uterus, and adipose tissue. From our results on sialic acid in the G fraction of blood and brain we found that almost 80% of the sialic acid was still unaccounted for.

Chromatography in EEN showed roughly equal amounts of NANA and NGNA in both fractions. There were also small amounts of material with Rf's of 0.2 and 0.56. Warren (17) has reported the presence of NGNA in rat vagina.

Snail: By analysis, snails showed 6 mg. % G NANA and 79 mg. % R NANA in dry tissue. On chromatography of 37 micrograms of G no NANA was found. Considerable material remained at the origin. There was a small amount of material with Rf about 0.3 which showed tailing from the origin.

The R fraction (125 micrograms) showed very intense spots at NANA and NGNA in solvent EEN. The spot at NANA was greenish-blue, however. When this sample was run in BPH for 57 hours instead of 24 hours the two spots were separated into two spots at the position of NANA and NGNA, and the blue spot was ahead of NANA. Because the solvent front had moved off the paper, the Rf's could not be calculated. Under ultraviolet light the NANA and NGNA spots were identical in color to the known samples of NANA. The two spots were not isolated and rechromatographed to determine definitely whether they were NANA and NGNA. The greenish-blue spot was lavender under ultraviolet light. Shikimic acid and quinic acid (18) have these color characteristics, but we did not determine

whether the unknown was either of these acids.

Sea urchin: The results on the Aristotle's lanterns of sea urchins were 5 mg. % for G NANA and 20 mg. % for R NANA in dry tissue. The concentration of G in the lipid fraction was higher than that obtained in mice, but the amount of lipid was much smaller, making the dry weight results much lower than for mice. Both G and R showed the same chromatography pattern. There were two spots, at 0.33 and 0.44, with somewhat more NGNA than NANA.

Sea anenome: There was no sialic acid in the R fraction in sea anenomes since the correction factor was 100 per cent. There were 4 mg. % in the dry tissue, however.

Chromatography of 77 micrograms of R in solvent EHN showed a spot at Rf 0.33. Most of the material present, however, had Rfs less than NGNA. There were at least three of these spots. In fraction G at 77 micrograms there was material from Rf 1.6 to 5.9 with perhaps six spots, including one at 0.33 and 0.44. The separation was poor, but a discrete spot was present at 0.65. Fifty micrograms of G in solvent EPH also showed discrete spots at both 0.33 and 0.44. There was a great deal of brown insoluble material at the origin as in the G fraction from snails.

Chiton: Chitons contained from 6 to 8 mg. % NANA in dry tissue; in fraction R, 77 micrograms, there was no NANA apparent. Instead there was tailing from the origin down to about an Rf of 0.35. The results were very similar to those of sea anenomes. The G fraction also showed tailing from the origin with perhaps six components including spots at 0.33 and 0.44. There was also a spot at 0.55 as for sea anenomes, but nothing at 0.65.

Clam: Clams contained 3 mg. % and 32 mg. % NANA in dry tissue for G and R fractions respectively. Chromatography of 125 micrograms of G showed a bright pink origin under ultraviolet light, and brownish color under

visible light. There was material from Rf of 0.07 to 0.48 including spots at 0.33 and 0.44. With 90 micrograms of R the results were similar to G.

DISCUSSION

Every animal tested apparently had sialic acid in the G fraction. In the brain tissue, where the identity was definitely established, the type of sialic acid was NANA. The sialic acid in both R and G fractions was similar for brain. There was no consistent relationship between NANA concentration and phylogenetic level of the nervous system. If the ganglioside sialic acid were the same constant for chordate brains then one would expect a decrease in the mg. % sialic acid in lipid along the phylogenetic scale from human to fish as the amount of cortex decreases with concomitant increase in white matter. White matter contains more lipid (19) and less sialic acid (9, 10). This should also be true on a dry weight basis as the size of the cerebrum decreases with respect to the cerebellum. The latter has been reported as having a NANA concentration similar to cerebral white matter (16). That NANA does not decrease is shown in the bar-graph of Fig. 5. The values for chordate brains cover a two-fold range, from 300 to 600 mg. %. These differences may be due to different amounts of di- and mono- sialogangliosides which have recently been isolated (20). The lipid-free residue values are from 330 to 560 mg. %.

There is also no systematic relationship between the ratio of R/G NANA on a dry weight basis in chordates. Human beings, turtles, snakes, frogs and fish have ratios above one, while the remainder are below one. As mg. % of the respective brain fractions, only rats, mice, and chickens have an R/G ratio below one. It appears that if the NANA concentration of G is from 500 to 600 mg. %, then the G values are higher than for the R fraction. The reverse appears to be true if the concentration of the G fraction is 300 mg. %. Whether these figures have any significance is not known.

In the whole animal tissues the concentration of sialic acid was less in the lower forms of animals when compared to mice except for the sea urchin (See Fig. 5). NANA appeared to be absent in snail and crab but NGNA was present in these and the other whole tissues tested. There were also other acidic compounds which were unidentified. In whole mouse, sea urchins and clams, the sialic acids of the R and G fraction were similar.

In chitons, sea anemones and snails they were not. Chitons and sea anemones resembled each other. The G fraction contained both NANA and NGNA, but in the R fraction only NGNA was present. Snails differed from all other samples in having a greenish-blue spot which was ahead of NANA on paper.

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Fig. 1. Hydrolysis of Bound N-acetylneuraminic acid in Rat Brain vs. Time

A. Time curve for ganglioside NANA as mg. % of total brain lipids (80° C, 0.1 N H₂SO₄)

B. Time curve for Residue NANA as mg. % of total brain lipid-free residue (80° C, 0.1 N H₂SO₄ for 75 min. and 2 hrs.; 90° for 65 min., 2, 3, 4, and 5 hrs.)

(All values corrected for interference)

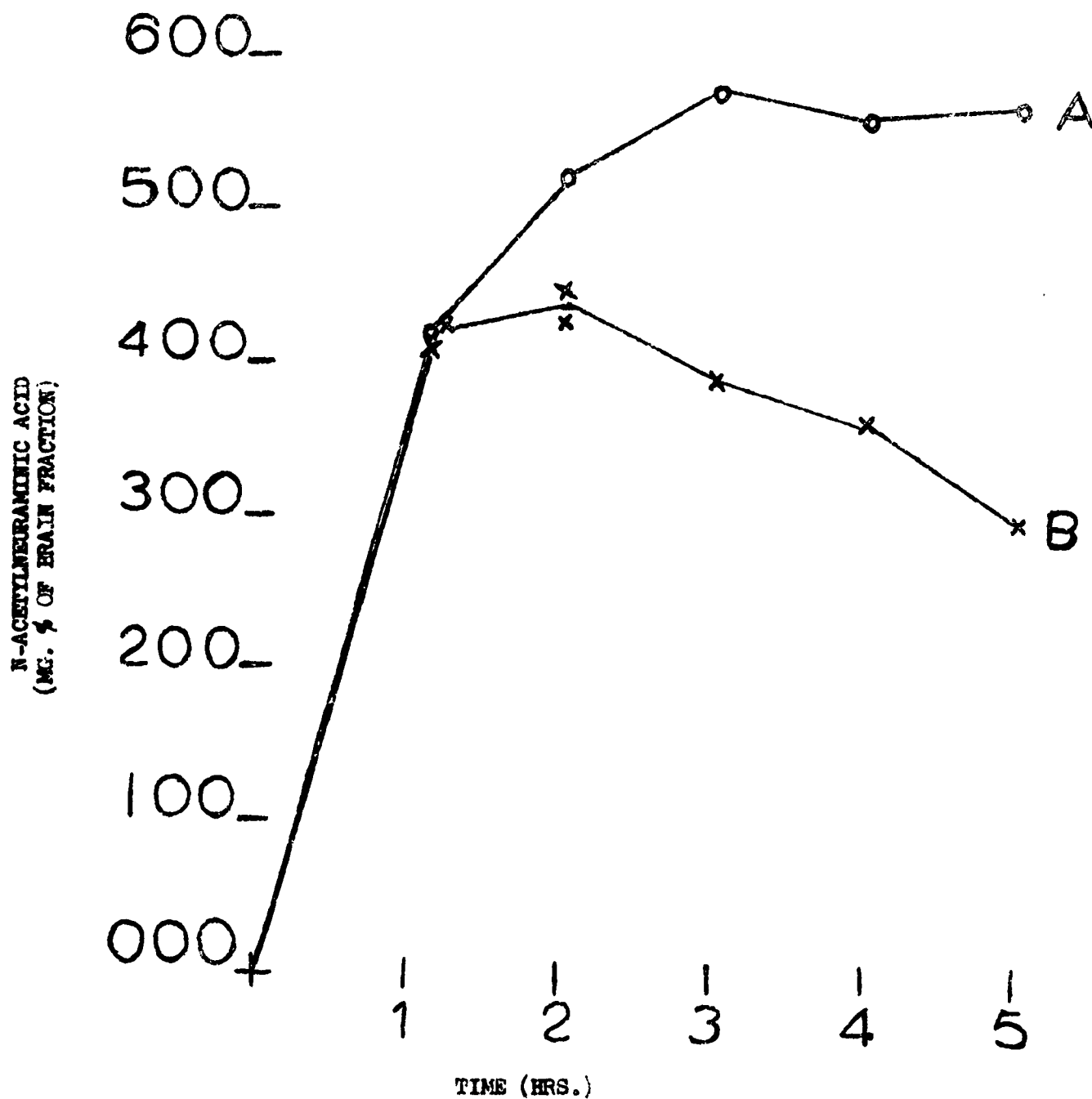


Fig. 2

Fig. 2. Comparison of bound N-acetylneuraminic acid (NANA) from rat brain Ganglioside and total lipid vs. time (0.1 N H_2SO_4 , $80^\circ C$)

A. Hydrolysis time curve for total lipid NANA (uncorrected)

B. Hydrolysis time curve for ganglioside NANA (corrected)

C. Same as curve A after correction for interfering absorption with maximum at 530 m μ

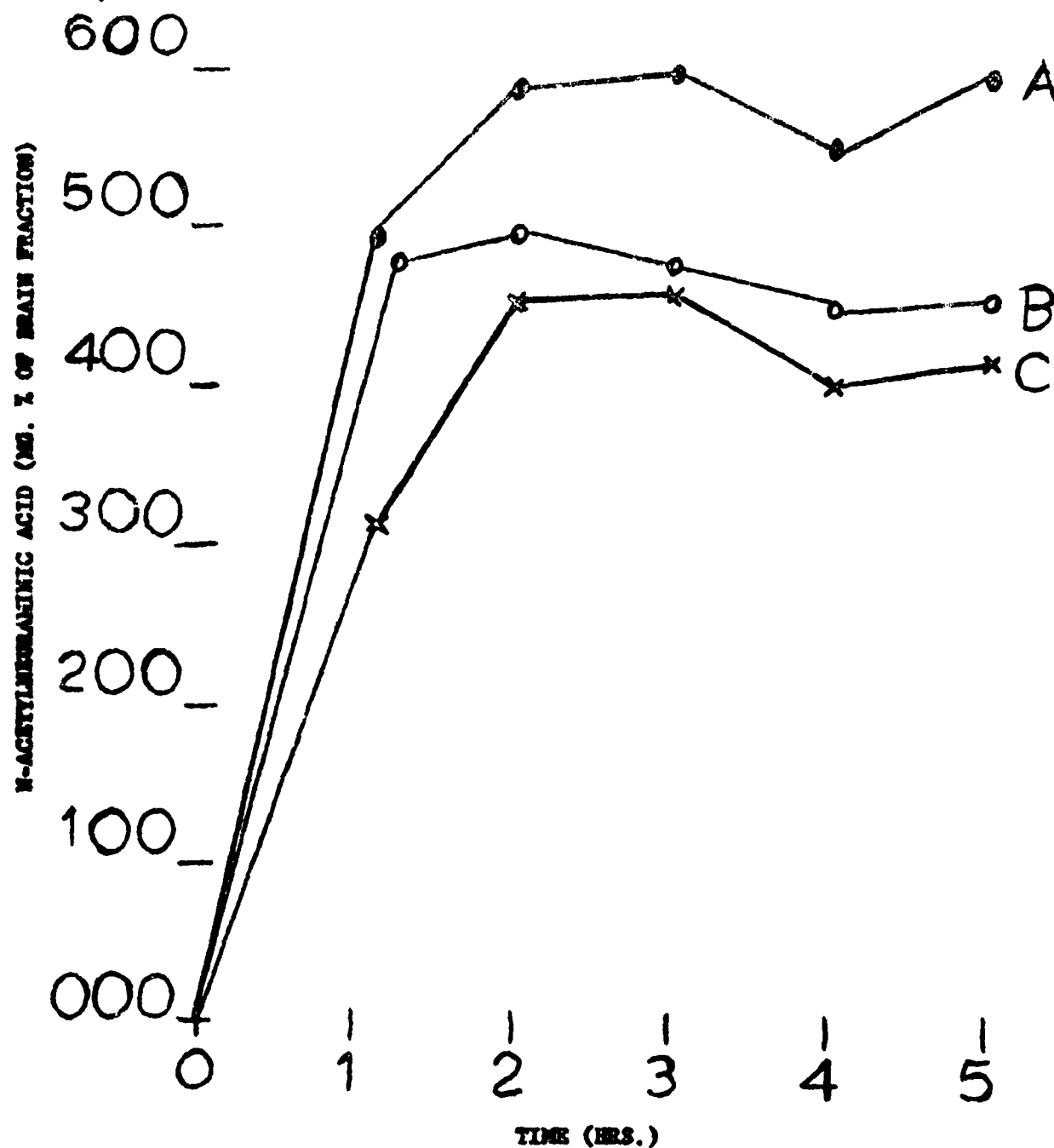


Table 1
The sialic acid content of brain fractions of animals
(calculated as N-acetylneuraminic acid)

animal	No. samples	%H ₂ O total brain	% Lipids		Mg. % Ganglioside			Mg. % Lipid-free residue		
			total solids	total brain	NANA total lipids	total solids	total brain	NANA total residue	total solids	total brain
Man	1	76*	57	14	311	178	43	527	225	54
Beef	1	80	61	12	342	207	42	375	148	30
Pork	1	83	62	10	315	195	32	360	137	23
Rat	7	79 \pm	51 \pm	11 \pm	510 \pm	256 \pm	57 \pm	405 \pm	201 \pm	46 \pm
	s.d.+	1	3	1	74	45	12	110	50	10
Rat (perfused)	5	80 \pm	52 \pm	11 \pm	513 \pm	265 \pm	54 \pm	450 \pm	218 \pm	45 \pm
	s.d.	1	2	1	31	28	4	44	32	8
Mouse	2	79 \pm	53 \pm	11 \pm	612 \pm	312 \pm	64 \pm	458 \pm	225 \pm	46 \pm
	s.d.	2	12	1	17	10	2	3	16	6
Chicken	3	80 \pm	50 \pm	10 \pm	570 \pm	286 \pm	59 \pm	415 \pm	205 \pm	42 \pm
	s.d.	1	5	1	48	12	5	8	15	1
Turtle	3	82 \pm	44 \pm	8 \pm	282 \pm	124 \pm	22 \pm	335 \pm	189 \pm	33 \pm
	s.d.	1	2	1	48	23	5	81	47	7
Snake	3	80 \pm	54 \pm	10 \pm	301 \pm	161 \pm	32 \pm	347 \pm	163 \pm	32 \pm
	s.d.	1	2	1	31	18	4	132	67	13
Frog	4	85 \pm	45 \pm	7 \pm	295 \pm	135 \pm	22 \pm	520 \pm	285 \pm	44 \pm
	s.d.	2	2	1	108	53	10	113	65	11
Fish	4 "	80 \pm	56 \pm	11 \pm	324 \pm	185 \pm	37 \pm	563 \pm	251 \pm	50 \pm
	s.d.	1	4	1	21	1	3	16	30	6
Crab	1	83	36	6	20	7	1	21	14	2

* value taken from reference 21

+ standard deviation calculated as $\pm wK_v$ where w = range and K_v is a constant from Table 1 of Dean and Dixon (8)

" 3 samples instead of 4 for the ganglioside sialic acid.

Table 2

The sialic acid content of whole tissues of various animals

(Calculated as N-acetylneuraminic acid - NANA)

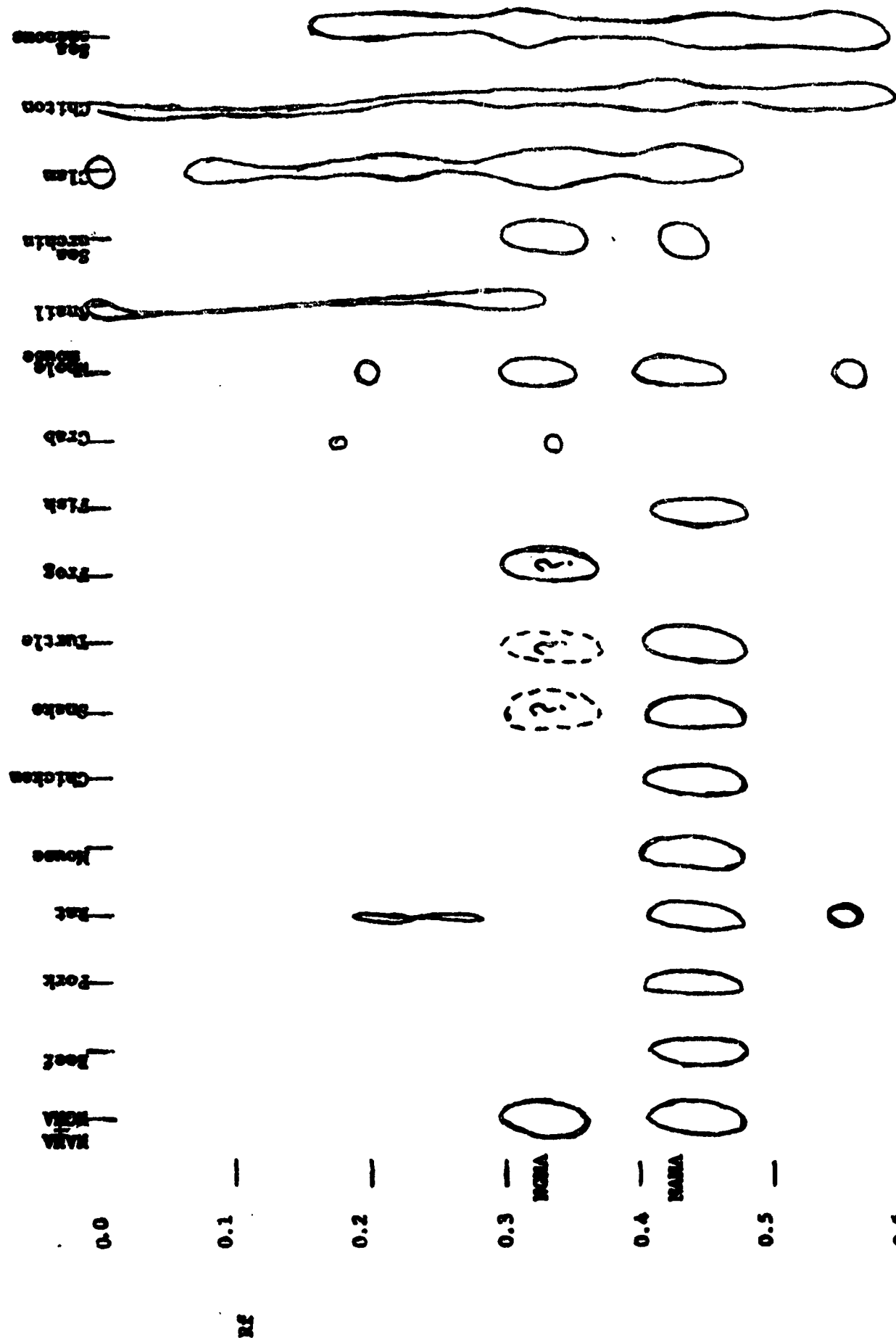
animal	No. samples	% H ₂ O % Lipids			Mg. % Ganglioside			Mg. % Lipid-free residue		
		total brain	total solids	total brain	NANA total lipids	total solids	total brain	NANA total residue	total solids	total brain
Mouse	1	73	33	9	66	23	6	120	79	22
Snail	2	83±	16±	3±	36±	6±	1"	83±	70±	12±
	s.d.*	1	2	4	6	2		27	21	2
Sea urchin	4+	50	5	3	80	5	2	21	20	11
(Aristotle's		±	±		±	±		±	±	±
lantern)	s.d.	5	1		8	1		6	5	3
Clam	2	79±	19±	4	17±	3±	>1	40±	32±	5±
	s.d.	1	1		7	1		14	11	2
Chiton	1	93	20	2	31	6	>1	10	8	1
Sea anemone	3	80±	28±	6±	16±	4±	>1	0		
	s.d.	1	10	2	9	2				

* Standard deviation calculated as for Table 1

" Where no s.d. is given it indicates that the value was less than 0.5

+ Calculated from 3 values for ganglioside NANA instead of 4.

Fig. 3. Paper Chromatography of Ganglioside sialic acids from brain and whole tissues of various animals



(Whatman No. 1; Solvent: Ethanol: H₂O: NH₃ (80:20:1))

Fig. 4. Paper Chromatography of Lipid-free Residue Sialic acids from brain and whole tissues of various animals

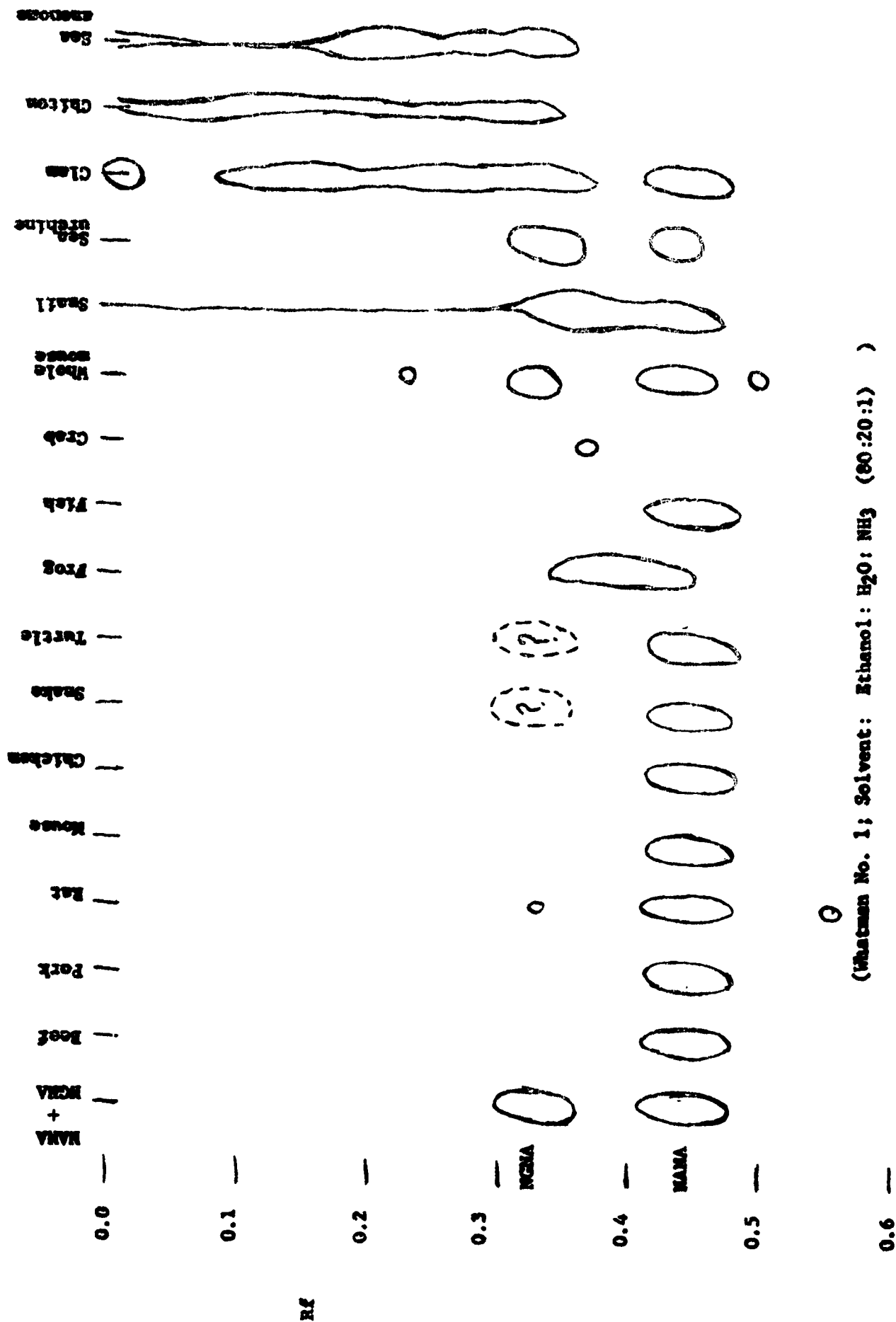
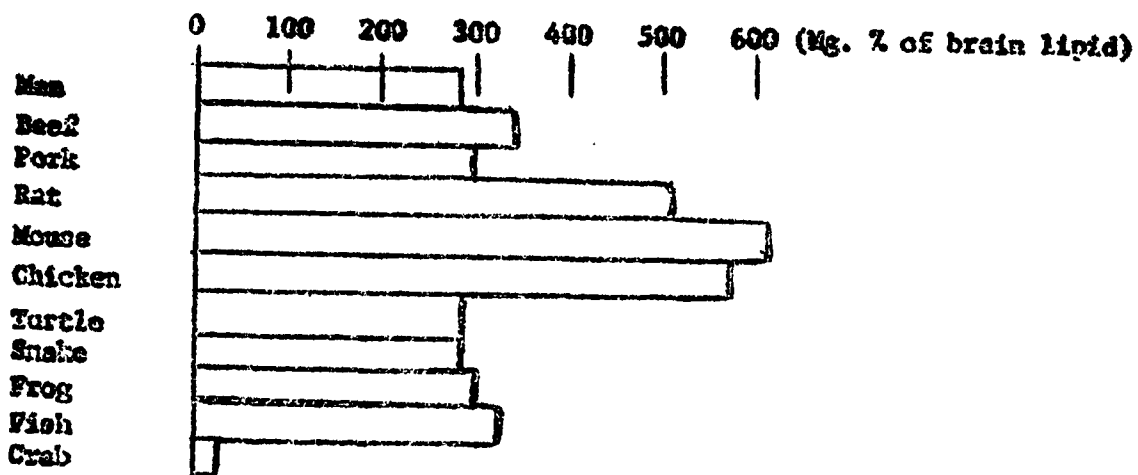
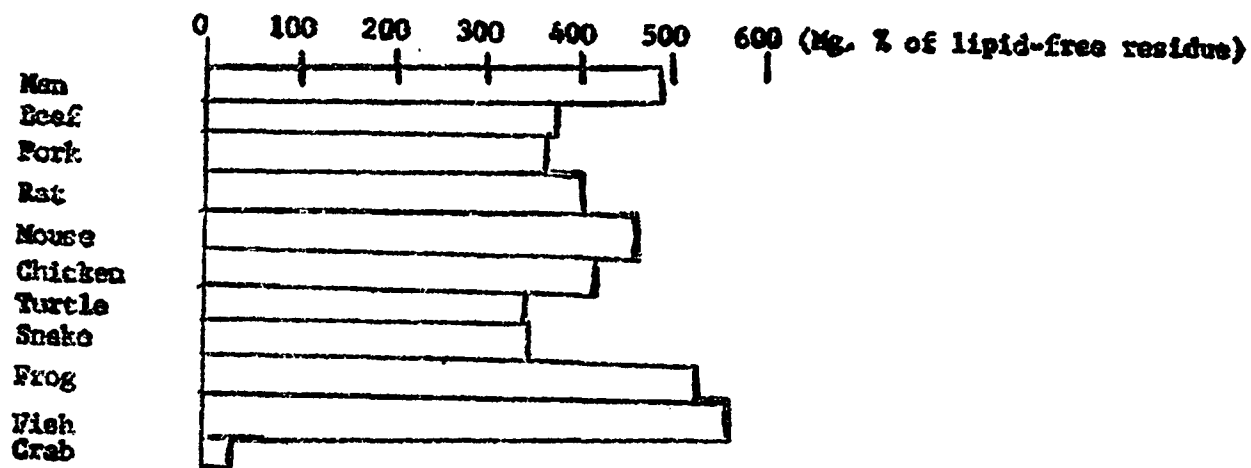


Fig. 5

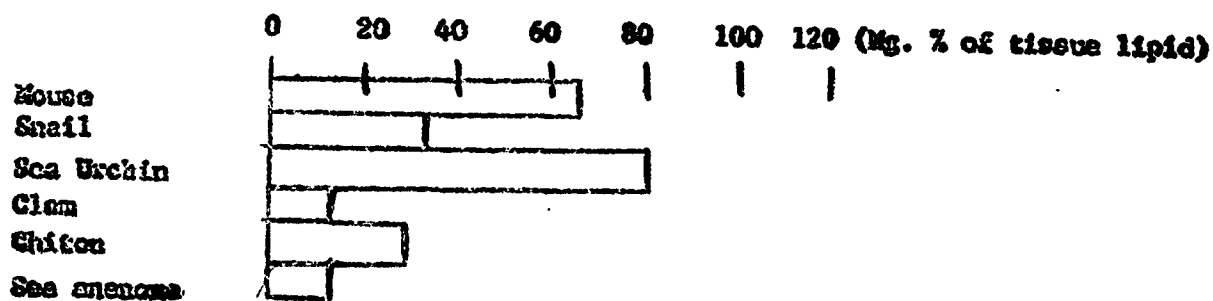
N-acetylneuraminic acid in the Brains and Tissues of Various Animals
BRAIN GANGLIOSIDE NEURAMINIC ACID (NANA)



BRAIN RESIDUE NANA



WHOLE TISSUE GANGLIOSIDE NANA



WHOLE TISSUE RESIDUE NANA

